# Inorganic Materials Biotechnology: A New Industrial Measurement Challenge

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Biotechnological processing of inorganic, heavy elements has only begun to emerge as we start to understand microbial strategies and mechanisms of heavy element transformations. Chemical speciation of key, diagnostic intermediates and products of bioprocessing in gas, liquid, and cellular phases, and on surfaces, is required to understand and optimize important reactions. Recent discoveries of microorganisms in metal-enriched thermal environments, and further investigations into production of exocellular metal transforming metabo-lites, offer exciting prospects for development of new technologies for strategic and precious materials recovery and processing.

## 1. Introduction

### 1.1 Inorganic Materials Biotechnology—Its Roots and Potential

Among the oldest of man's technologies, biotechnology has lately captured scientific, commercial, and public attention for the wide scope of organic materials such as pharmaceuticals, foodstuffs, or commodity chemicals, and improved energy economies, it presages. Basically, biotechnology "... involves the use of living organisms to make commercial products and includes three major areas of research effort: recombinant DNA methodology, monoclonal antibody techniques, and bioprocessing ..." [1][^1]. The recent

[^1]: Figures in brackets indicate literature references.

challenges and opportunities perceived for biotechnology depend upon a clear and reliable measurement base at the level of molecular biology, and hence increasingly must involve novel contributions from many chemical disciplines as well.

Not so familiar to casual onlookers and non-specialists is the fact that biotechnology deals with or more than organic materials comprised of a few light elements such as carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus; it deals with the majority of the remaining 90-odd elements that also influence our biosphere. However, inorganic materials bioprocessing is receiving attention from many industries where microorganisms are increasingly perceived as potential low-energy routes to recovering metals from ores, upgrading of ores and fossil fuels prior to conventional processing and combustion, and toxic and/or precious metal precipitation and recovery in waste streams. The po-

tential of this biotechnology was addressed by academic, government, and industrial representatives at the recent Workshop on Biotechnology for Mining, Metal-Refining and Fossil Fuel Processing Industries [2] at Rensselaer Polytechnic Institute, Troy, NY (May 28–30, 1985). It is now clear that major sectors in North American mining technologies are moving rapidly to further develop biotechnological processing, including, for example CANMET (Canada Centre for Mineral and Energy Technology) [2] and the participation of the U.S. National Laboratories investigating "leapfrog" technologies for the steel industry [3]. One such technology would be bioprocessing of iron ores for removal of impurities or waste bioprocessing.

Investigations into the range and potential for commercial microbial transformations of heavy elements are just beginning. This paper therefore emphasizes inorganic bioprocessing, its industrial potential, and the measurements needed to characterize, monitor, and apply the inorganic reactions that microorganisms perform.

## 1.2 Environmental Perspective of Inorganic Materials Biotechnology

The lighter "organic" molecule-forming elements (C,H,O,N,S,P) make up the bulk of cell constituents and thus are essential nutrients for cells. However, many "inorganic" or heavy elements, including both metals and metalloids are essential in biological processes, often as enzyme cofactors, but in some cases as energy sources and electron acceptors in respiration [4]. Still other heavy elements are toxic and have no known metabolic function, such as Hg or Cd. Organisms which detoxify (by oxidizing, reducing, complexing, alkylating) these elements are often found associated with metal ore deposits, industrial waste discharges and other special and unusual environmental sites including, geothermal springs in terrestrial volcanic locales or geothermal sea-floor vents recently reported at great ocean depths [5]. Unexpectedly rich growths of microorganisms isolated from these metal-rich regions display capacities for heavy metal transformations even at high temperatures and pressures, implying tremendous possibilities for bioprocessing.

Microbial processing of metals and metalloids also occurs in less severe environments as evidenced by the burgeoning literature describing natural biotransformations of many elements [4]. At this point, our state of knowledge is incomplete regarding the range of beneficial as well as inimical

transformations of many heavy elements. From an environmental viewpoint, this issue is, and must remain, a vital R&D component of monitoring and regulation. For example, the public health disasters in Japan two decades ago resulting from "Minimata Disease," or methylmercury poisoning, of a general seacoast population eating contaminated fish were exacerbated by underlying chemical and bacterial transformations of industrial mercury discharges into marine sediments and subsequent transmission into food webs [6]. Nonetheless, the same basic understanding of metals biotransformations and the related measurement methodology also supports promising new ventures in "inorganic" biotechnology.

Following the Minimata disaster, work in many laboratories, including ours at NBS, showed the prevalence of environmental mobilization by biotransformations of mercury and other heavy elements in polluted sediments [6]. Recent reports show that microorganisms may possess metal-induced resistance to metals and antibiotics [7,8]. Several elements besides mercury have been implicated in this way, including arsenic [9], silver [10], and cadmium [11]. Hence, applications of such results growing out of environmental and health research bears equally great promise for inorganic bioprocessing designs and applications.

## 1.3 Mechanisms of Metals Biotransformations

Since extra-chromosomal components of bacteria, called plasmids, have been demonstrated to confer new enzymatic capabilities in cells for highly effective metal- and metalloid-processing [12], and these plasmids can be genetically manipulated within current biotechnology, the earlier information on environmental impacts of such elements may now find new utility in the design and application of genetically-modified bacteria capable of industrially important processes. Plasmid mediated metal transformation often serve to detoxify metals. However, some microorganisms enzymatically oxidize heavy elements such as Fe(II), Sb(III), or U(IV) as energy sources. In certain bacteria, electrons from these reduced metals are passed to various electron acceptors spanning the cytoplasmic membrane. This generates a proton motive force across the membrane which drives ADP phosphorylation to ATP [13]. Table 1 summarizes a number of reactions for those microorganisms thus far studied and offers two examples of potential energy sources, but it should be regarded in light of the vast potential for useful reactions of many other heavy elements thus far unexplored.

Table 1. Examples of heavy elements used as energy sources by bacteria.

| Metal Bioprocess | Terminal $e^-$ Acceptor | Organism | Ref. |
|---|---|---|---|
| $Fe^{2+} \rightarrow Fe^{3+}$ | $O_2$ | T. ferrooxidans | 13 |
| $Sb^{3+} \rightarrow Sb^{5+}$ | $O_2$ | S. senarmontii | 14 |
| $U^{4+} \rightarrow U^{6+}$ | $O_2$ | T. ferrooxidans | 15 |
| $Cu^+ \rightarrow Cu^{2+}$ | $O_2$ | T. ferrooxidans | 16 |

Examples of reactions that potentially yield enough energy for ATP synthesis, but not yet demonstrated with organisms.

| | | |
|---|---|---|
| $Co^{2+} \rightarrow Co^{3+}$ | $O_2$ | 17 |
| $As^{3+} \rightarrow As^{5+}$ | $O_2$ | 18 |

The paucity of knowledge of such "inorganic" biochemistry has and will remain subject to progress with measurement state of art. Reliable methods for characterization of microbial metal transforming processes are needed both for understanding mechanisms and, ultimately, for process monitoring in the commercialization of such element-transforming processes [19].

## 2. Measurement Requirements and Prospects for Inorganic Materials Biotechnologies

Though not widely described, there already exists in the United States and elsewhere industrial use or practical demonstrations of microbial processing of metal ores *in situ* and sequestering of heavy metals from waste streams. Table 2 provides examples in recent use along with the key metal

Table 2. Examples of commercial applications of metals bioprocessing.

| Application | Industry | Metal Product | Ref. |
|---|---|---|---|
| Ore leaching | | | |
| Cu | Mines in W. USA | $Cu^{2+}$ | 20 |
| U | Mines in Canada, USA | $U^{6+}$ | 21 |
| Total metal precipitation | | | |
| Pb, Zn | Mines in Missouri | oxides, sulfides | 22 |
| U* | Nuclear waste effluents | $UO_2^{2+}$ | 23 |
| Precious metal recovery | | | |
| Ag* | Photographic effluents | $Ag^\circ$ | 24 |
| Ag, Au* | Pyritic gold, silver ores | $Ag^\circ$, $Au^\circ$ | 25 |

* Laboratory scale investigations for feasibility of bioprocessing.

products. The translation of laboratory demonstration to cost-effective field processing will largely depend upon the reliability and sensitivity of real-time monitoring, as well as the measurement capa bility for element-specific quantitation. Where field use is already practiced, effects of potential rate limiting toxicants and means for process optimization have yet to be fully investigated. Future developments will also likely include bioprocessing of ore concentrates under controlled conditions. In such processes, container materials must withstand corrosive biogenic agents and possibly elevated temperatures and pressures.

### 2.1 Process Options

Experience in inorganic bioprocessing to date indicates [26] that three major separate or concerted routes are effective in transforming, isolating, and concentrating desired heavy elements. These are: 1) chemomechanical separation and concentration; 2) solubilization (and redox); and 3) direct cellular uptake or accumulation.

**2.1.1 Surface Pretreatments Using Biomodification.** Fossil fuel upgrading employing microbial cell cultures has been used to remove pyritic sulfur, that is iron sulfide constituents, from coals either by direct solubilization of the mineral component [20], or by rendering the pyrite hydrophilic through biotransformation followed by oil agglomeration [27]. The surface-modifying microorganisms are known to produce phospholipid wetting agents or surfactants [28] which enable subsequent mechanical treatments to dissemble and separate otherwise tightly bound composite matrices.

The bacterium *Thiobacillus ferrooxidans* has been used in preoxidation treatments of gold and silver ores [29]. These precious metals often occur finely disseminated in iron pyrites which *T. ferrooxidans* oxidizes readily. Preoxidation of such ores improves gold and silver recoveries by subsequent conventional extraction methods.

**2.1.2 Mineral Solubilization by Cells and Cell-Free Metabolites.** Pyrite ($FeS_2$) oxidation by *T. ferrooxidans*, catalyzed by direct enzymatic attack and by biogenic acidic ferric sulfate solutions, is accelerated by a factor of up to $10^6$ over the abiotic rate [30] with important industrial and environmental consequences. Microbially catalyzed pyrite oxidation is responsible for the production of acidic coal mine drainage. However, the acidic ferric sulfate solutions generated by this oxidation also solubilize metal sulfides in mining operations employing bioleaching.

Clear evidence was recently presented showing severe corrosion of structural iron artifacts by anaerobic bacterial production of as yet unidentified metabolites transmitted in solution or in respirant atmosphere [31].

Other evidence for a number of metabolic agents, themselves either of organic or inorganic nature, suggests that microbial solubilization of minerals is naturally widespread or can be exploited in chemical plant scale. Some of the key agents released by living cells may solubilize otherwise commonly highly refractory substances such as mineral ores, ceramics, and bulk metals as summarized in table 3. However, most of the reactions reported (table 3) have not identified the specific molecular entities produced by cells, nor quantitated the mechanistic path of solubilization. Recent preliminary work at NBS illustrates one mechanism of mineral sulfide solubilization by the pervasive algal metabolite methyl iodide. This metabolite appears to break metal-sulfur bridges by oxidative addition at sulfur or metal centers, weakening the crystal lattice and eventually allowing the metal to dissolve.

Production of powerful biogenic solubilizing agents can cause problems in process containment. Studies on corrosion resistance of materials to these metabolites are warranted.

**2.1.3 Membrane and Cytoplasmic Uptake and Selective Precipitation of Elements.** As ultratrace chemical speciation methods have gained favor and applicability, evidence has grown to support novel use of molecular topology arguments as structure-activity predictors for cell uptake of several metal species, notably tin [39]. Transmission of certain chemical forms of metals and metalloids across membranes occupies much attention of toxicologists and molecular geneticists [40] but much

additional research is needed to complete our understanding of superficial membrane complexation of metal and metalloid ions, their entry into cell interiors and their ultimate fate. Nonetheless, progress has been made in describing bacterial uptake-transmission of isostructural, isoelectronic, and isosteric agents, as illustrated by the arsenate-phosphate [9], divalent cations [41], organotins [42] cases. Far more difficult will be the establishment of underlying biochemical factors that control the rate and extent of precipitation and morphology of those elements and their compounds that actually agglomerate in diffuse or specific sites within living cells. The case of *Aquaspirillum magnetotacticum*, a widely distributed aquatic bacterium, is of special interest because of its highly efficient and selective uptake of Fe(III) from solution, concentration, and derivatization to pure magnetite ultramicrocrystals aligned within the cell major axis [43]. The commercial consequences of understanding the mechanisms involved in such biosynthesis of hyperferromagnetic ultraparticles are significant especially in view of prospects for other strategic metals compounds.

### 2.2 Element-Specific Detection, Imaging, and Quantitation

From the foregoing discussion it is evident that progress is needed in defining tell-tale chemical reactions and their rate-determining participants within the controlling biological matrices. Understanding these processes requires measurements with a high degree of element- and species-specificity and extremely low detection limits characteristic of living cells. These general requirements for ultratrace analysis of chemical speciation of heavy elements undergoing con-

Table 3. Examples of metal solubilization by low-molecular weight extracellular metabolites.

| Metabolite | Source Organism | Substrate | Product | Ref. |
|---|---|---|---|---|
| Methyl iodide | Marine algae | Metal sulfides | Soluble metal | 32 |
| | | SnS | $CH_3SnI_3$ | 33 |
| Siderophores | Bacteria | $Fe(OH)_3$ | Iron chelates | 34 |
| Unidentified | Bacteria | Au | Soluble gold | 38 |
| Unidentified | $SO_4^-$ reducing bacteria | $Fe^0$, steel | Soluble Fe | 31 |
| | | brass | Soluble Cu | 31 |
| Unidentified | *Penicillium* | $Fe^0$, steel | Soluble Fe | 35 |
| | | Chalcocite ore | Soluble Cu | 36 |
| Methylcobalamin | Bacteria | Pb, Sn oxides | Soluble Pb, methyltin | 37 |
| | | | | 37 |

trolling bio-transformations have received recent review [44], but several basic points merit emphasis in addition to common features of preconcentration, molecular separation, and selective detection within meaningful time frames. Chemical speciation of important bioprocess products may be required in solution, gas phase, on surfaces, or intracellularly.

**2.2.1 Element Speciation in Cellular Media.** Our laboratory has developed chromatographic systems (HPLC, GC) coupled with element-selective detectors for speciation of metal-containing biogenic molecules in gas or liquid phases at ultratrace (pg-ng) levels. Volatile forms of metals have been speciated by GC-AA [45,46], or by GC with a flame photometric detector made selective for the element of interest by different flame gas flows and optical interference filters [47]. Solvated forms of metals can be speciated using liquid chromatography coupled with atomic absorption [48] or epifluorescence microscope (using fluorescent ligands) [49,50] detectors.

**2.2.2 Element Speciation at Substrate or Cell Surfaces.** Methods for chemical speciation on surfaces are not as well developed as for liquid or gas media. It may be possible to dissolve or extract the material of interest from cells and perform analyses described above. We were able to measure the accumulation of tributyltin species on estuarine bacteria by HPLC-GFAA analyses of methanol extracts of cells [51]. More promising is the potential for using element- and chemical species-selective fluorescent ligands for imaging these molecules on cell or substrate surfaces. Recent work in our laboratories suggests that inorganic and organotin species accumulation on bacterial cells can be monitored using a fluorescent ligand, 3-hydroxyflavone with detection by epifluorescence microscopy [52] in which the microscope isequipped with a monochromator and photometer for measuring fluorescence emission intensities at appropriate wavelengths. Another surface analysis technique, Fourier transform infrared spectroscopy, is promising for study of biological reactions at surfaces. We have begun to apply FTIR to analyze biotransformation of coal leading to removal of specific undesirable functional groups [53]. Others are just beginning to study biofilms with this method [54].

**2.2.3 Element Speciation Within Cells.** Intracellular microbial processing products may be studied by breaking cells (sonication, pressure disruption, chemical treatments) and analyzing their contents by methods described above. Electron microscopy with x-ray microanalysis is useful for spa-

tial characterization of elements in cells. However, nondestructive methods for speciation of process intermediates or products occurring intracellularly are more difficult. Nevertheless, recent progress in nuclear magnetic resonance spectroscopy has led to the application of this nondestructive technique to real-time monitoring of cellular metabolic processes [55].

Early work in the field of small angle neutron scattering (SANS) techniques for investigating protein structure is being undertaken [56]. However, the potential for studying intracellular metabolic processes has not been investigated. A particularly attractive model system for studying the application of SANS to biological transformations of materials is *Aquaspirillum magnetotacticum*—a magnetotactic bacterium that intracellularly deposits pure magnetite crystals on the order of 500 Å diameter [43]. We have undertaken preliminary experiments using SANS to measure magnetite particle formation rates in cells and to better understand how the cells can perform such an exact synthesis, in terms of size and purity, of these hyperferromagnetic particles.

## 2.3 Chemical Speciation and Process Monitoring

Materials bioprocessing will increasingly require monitoring of intermediates and products diagnostic of proper or optimal conditions. A better understanding of the enzymatic transformations of toxic metals by metal resistant process organisms is also needed. This includes analyzing metal speciation in solution and gas phases and bound to cell surfaces or precipitates. Many of the measurement techniques described in the previous sections may find application for such requirements. For example, the presence of toxic metals, such as Hg, in metal ore bioleaching systems has been suspected of inhibiting the beneficial action of the process organisms. Results in our laboratory and elsewhere showed that mercuric ions at concentrations as low as 10 $\mu$g/L inhibit iron oxidation by *Thiobacillus ferrooxidans* [57]. Iron is an energy source for the organism, and is added to the growth medium at 10-40 g/L levels. However, we have isolated a mercury-resistant strain of this organism and, using a gas chromatograph coupled with a flameless mercury atomic absorption detector, determined that the organism reduced $Hg^{2+}$ to $Hg^{\circ}$ which was rapidly volatilized from the growth medium [57]. Thus, it is evident that mercury resistant strains of this species can be selected for by mercury in the environment. In collaboration with molecular biologists at Washington University, we characterized

the mechanism of reduction and found that the organism produces a heat-stable, intracellular mercuric reductase enzyme that uses reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor [58]. With this information, a selectable genetic marker is now available for others to study in more detail, using recombinant DNA techniques, the genetics of this important industrial organism.

## 3. Current NBS Activities in Inorganic Materials Bioprocessing

Exciting new prospects for inorganic materials bioprocessing are presented by some of the recently discovered thermophilic bacteria (fig. 1). These include *Sulfolobus* [59] and other bacteria [60] discovered in hot springs and ore dumps, and the extreme thermophiles associated with volcanic activity in shallow [61] and deep [62] ocean depths where water exists in liquid form above 100 °C. These metal-enriched vent waters support novel microorganisms which potentially are applicable to metals bioprocessing since biological and chemical reactions are accelerated under increased temperature and pressure. We have recently begun a pro-

ject to evaluate the bioleaching and recovery of strategic metals, in part using thermophilic microorganisms to accelerate reactions. In addition to high temperature (50–100 °C) studies at ambient pressures, a high pressure-temperature bioreactor will be employed to study metal bioprocess rates at pressures up to 250 atmospheres and temperatures of up to 200 °C.

Containment and process monitoring becomes difficult under the above conditions, especially when low pH and corrosive metabolites are involved. Conventional steels and plastics undergo deterioration under these extreme conditions [63] and alternative materials such as advanced ceramics will be required for any future industrial applications. The economics of metal processing under such conditions will be dictated by the value of the material and the enhancement or uniqueness of microbial processing compared to chemical or physical techniques.

The processing of metals using exocellular microbial metabolites is also under investigation. We found bulk metals and metal sulfides to be dissolved by methyl iodide (table 3), a common algal and fungal metabolite. Currently, we are examining processing of ores by MeI and other biogenic metabolites. It may be possible to generate such

**THERMOPHILIC MICROORGANISMS**

organisms growing at ≥50 °C

these environments usually associated with volcanic phenomena

but also

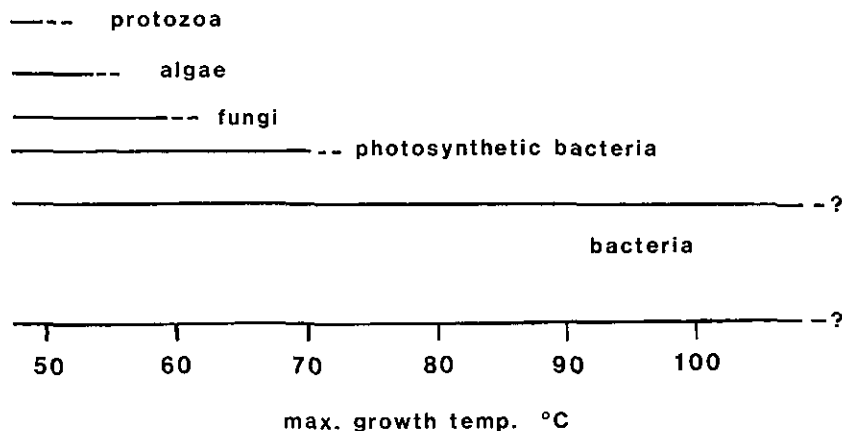metal ore leach dumps
coal refuse piles
decaying organic matter

— ▪ ▪▪ protozoa

——— ▪▪▪ algae

——————— ▪▪▪ fungi

————————————— ▪▪ photosynthetic bacteria

———————————————————— ▪ ?

bacteria

———————————————— ▪ ?



| | | | | | |
|---|---|---|---|---|---|
| 50 | 60 | 70 | 80 | 90 | 100 |

max. growth temp. °C

Figure 1–The relationship between maximum growth temperatures and various genera of microorganisms are compared, along with common environments supporting their growth. Newly discovered thermophilic bacteria have been shown to grow at 115 °C under pressure [61]. Unconfirmed reports of microbial growth at 250 °C and 200 atmospheres pressure have recently appeared [62].

metabolites in remote bioreactors and apply the solutions to the material to be processed.

We are also investigating prospects for using microorganisms for synthesis of selected metal forms. For example, certain thermophilic algae precipitate nickel as the sulfide exocellularly [26], magnetotactic bacteria produce magnetite crystals from soluble iron [43], and *Thiobacillus* and other resistant bacteria reduce mercuric ions to elemental mercury. The use of such organisms to produce desired molecular forms of strategic or precious metals is under investigation in our laboratories.

## 4. Conclusions

Man has used biotechnology for centuries, initially for fermentations and more recently in the food, pharamaceutical, and medical fields. How-

ever, we are only beginning to understand how to apply microorganisms for the recovery and processing of inorganic materials for commercial and technological applications. Our knowledge is incomplete in understanding mechanisms of microbial interactions with inorganic or heavy elements which may be transformed as energy sources, electron acceptors in respiration, or as toxic agents. Chemical speciation of such elements in aqueous, gaseous, or cellular media is essential in understanding mechanisms of biotransformations. Surface analysis or imaging is important because many biotransformations of these heavy elements involve reactions of insoluble substrates or precipitation. Newly discovered microorganisms in metal-rich thermal environments offer exciting prospects for novel and accelerated bioprocessing.

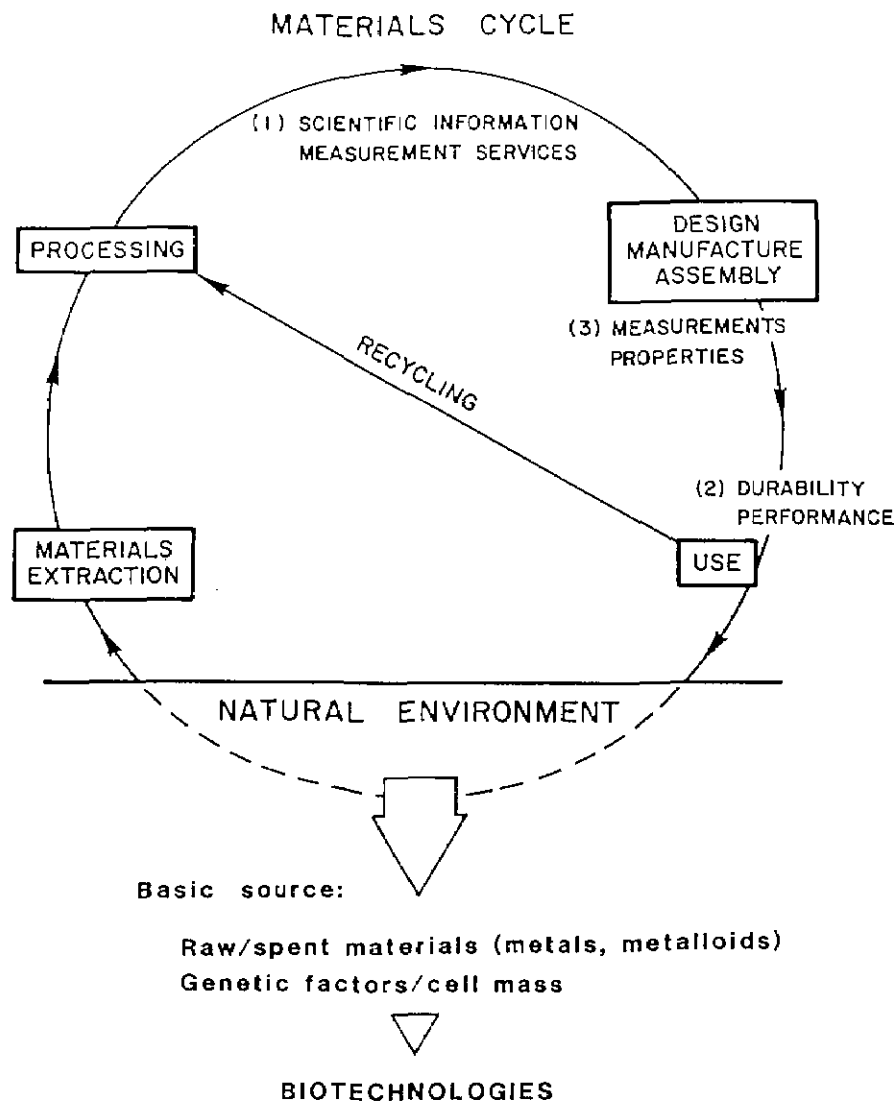In summary, a materials cycle is presented (fig. 2) which illustrates the potential microbial role in



MATERIALS CYCLE

(1) SCIENTIFIC INFORMATION
MEASUREMENT SERVICES

PROCESSING

DESIGN
MANUFACTURE
ASSEMBLY

RECYCLING

(3) MEASUREMENTS
PROPERTIES

(2) DURABILITY
PERFORMANCE

MATERIALS
EXTRACTION

USE

NATURAL ENVIRONMENT

Basic source:

Raw/spent materials (metals, metalloids)

Genetic factors/cell mass

BIOTECHNOLOGIES

Figure 2-The conventional "cross-cutting" features of measurements in relation to materials design, processing, use and recycling are here defined in terms of their frequent natural environmental interactions. Thereby, a coherent picture of new materials innovation and acquisition emerges in terms of biotechnological options.

acquisition and disposition of materials (the lower portion of the circle). We have shown the importance of and potential for microorganisms in materials extraction and for recovering and recycling of heavy elements from waste or process streams. Also, future developments in processing elements into selected forms appears promising.

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